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Running title: El-Sayed et al.: Kairomone for *Lobesia botrana*

Leafroller-induced phenylacetonitrile and acetic acid co-attract adult *Lobesia botrana* in European vineyards

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Abstract We have recently identified unique caterpillar-induced plant volatile compounds from apple leaves infested with various leafrollers species. Subsequently, in various field tests, a binary blend of phenylacetonitrile + acetic acid or 2-phenylethanol + acetic acid was found to be attractive to a range of tortricid leafrollers species (Tortricidae: Tortricinaei) in the Southern and Northern Hemispheres. In this work, the caterpillar--induced plant volatiles from the apple-leafrollers system were tested in two European vineyards in Spain and Hungary for the attraction to the grape fruitivore *Lobesia botrana* (Tortricidae: Olethreutinae). As seen for Tortricinaei species, a binary blend of phenylacetonitrile + acetic acid was the only combination that significantly attracted more male and female *L. botrana* to traps, compared with than acetic acid alone or blank traps. The cCatches in traps baited with caterpillar-induced plant volatile compounds (benzyl alcohol, 2-phenylethanol, indole, and (*E*)-nerolidol plus + acetic acid were not significantly different from captures in traps baited with acetic acid alone. Catches of significant numbers of male and female moths support an optimistic future for new applications in female tortricid surveillance and control based on combinations of kairomone compounds, emanating from larval-damaged foliage.

Keywords: kairomone, phenylacetonitrile, acetic acid, European grapevine moth,; *Lobesia botrana*

Introduction

Of the more than 40 species of Tortricidae that have emerged as targets of pest management research using pheromones, a number have also been targeted with official control or eradication programs (Suckling et al. 2016). Among these species is the European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae: Olethreutinae), which is a major pest in vineyards in 51 countries around the world (CABI 2018). A sex pheromone is currently being used to monitor and control ~~of~~ *L. botrana*, but female attractants have been long sought for the EGVM (von Arx et al. 2011)~~,~~ as a monitoring tool in vineyards under mating disruption, or for surveillance and detection outside its geographic range. With the growth in global trade, such species are expanding their geographic range, and new incursions regularly occur in different parts of the world. Currently, this pest has ~~been~~ become established in Chile and Argentina, while it was eradicated from California in 2016 (ref), and is on the watch list of invasive species in New Zealand and Australia.

Apple trees uniquely release several compounds, including acetic acid, benzyl alcohol, phenylacetonitrile, indole, 2-phenylethanol and (E)-nerolidol, when infested by larvae of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker); the eye-spotted bud moth (ESBM), *Spilonota ocellana* (Denis & Schiffermüller); the obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), and the pandemis leafroller moth (PLR), *Pandemis pyrusana* (Kearfott) (Suckling et al., 2012; El-Sayed et al., 2016; El-Sayed et al., 2018). Recently, El-Sayed et al. (2016) found that a binary blend of the three HIPVs, phenylacetonitrile + acetic acid and 2-phenylethanol + acetic acid attracted a significant number of

conspecific male and female adult LBAM in New Zealand. Further investigation with other leafrollers (Tortricidae) in North America, including the ESBM, OBLR, and PLR revealed similar responses. Male and female adults of ESBM were most attracted to a blend of phenylacetonitrile + acetic acid, while male and female adults of OBLR and PLR were most attracted to a blend of 2-phenylethanol + acetic acid (El-Sayed et al., 2016; Suckling & El-Sayed, 2017; El-Sayed et al., 2018), all of which are herbivores of apple in the subfamily Tortricini.

Comentado [CG1]: relocate this?

In this work, we hypothesized that ~~because many leafrollers will attack~~ grape leaves and ~~infested~~ grapes ~~might~~ attacked by leafrollers may release HIPVs compounds similar to ~~the compounds~~ those released by apple infested with leafrollers, and that the EGVM might have the ability to detect and respond to these compounds. Accordingly, we have extended our field trials and tested the HIPVs that were identified in the apple-leafroller~~s~~ system (Suckling et al., 2012, El-Sayed et al., 2016; El-Sayed 2018) in two European vineyards for the attraction of male and female EGVM.

Materials and Methods

Chemicals

The chemical purity of the standards used in the field experiments was as follows: Glacial acetic acid (99%), benzyl alcohol (99%), phenylacetonitrile (99%), 2-phenylethanol (99%), indole (99%), and (E)-nerolidol (98%, mixture of cis and trans). Glacial acetic acid was stored at ambient temperature while all other compounds were stored at -20°C until used. All compounds were purchased from Sigma-Aldrich (MO, USA).

Field Experiments

The first field trapping experiment was conducted in a grape vineyard (var. Tempranillo) in Verdú (41°35'46.3"N - 1°05'16.7"E) Lleida, Spain from 30 July - 27 August 2013. White delta traps (215 mm long × 200 mm wide × 100 mm tall, 340 cm2 adhesive base area, Opennatur, Lleida, Spain) ~~Size colour delta traps (source, Spain)~~ were used in this trial. The first field trapping experiment was conducted to investigate various blends of HIPVs identified in the infested apple trees and acetic acid (El-Sayed et al. 2016). Loadings of the nine HIPVs blends were prepared as follows: 1) 100 mg phenylacetonitrile + 3 mL acetic acid; 2) 100 mg indole + 3 mL acetic acid; 3) 100 mg (E)-nerolidol + 3 mL acetic acid; 4) 100 mg benzyl alcohol + 3 mL acetic acid. A trap baited with 3 mL of acetic acid alone and a blank lure was used as a control~~s~~.

The second field trapping experiment was conducted in a grape vineyard (var. Blue Frankish) in Novaj (47°50′06″N - 20°29′03″E) Heves county, Hungary from 22 August – 13 September 2017. Transparent delta traps (CSALOMON® RAG, Plant Protection Institute CAR HAS, Budapest, Hungary) with 10 x16 cm sticky insert were used in this trial. The second field trapping experiment was conducted to investigate various blends of HIPVs. Loadings of the xx HIPVs blends were prepared as follows: 1) 100 mg phenylacetonitrile + 3 mL acetic acid; 2) 100 mg 2-phenylethanol + 3 mL acetic acid; 3) 100 mg benzyl alcohol + 3 mL acetic acid. A trap baited with 3 mL of acetic acid alone and a blank lure was used as controls.

In both trials, for each treatment, 100 µL of the undiluted chemicals were pipetted into a 5 × 5 cm polyethylene sachet (Masterton, New Zealand) with a

wall thickness of 100 µm containing a rectangular piece of wool felt (4 × 2 cm^2). The acetic acid dispenser was made by pipetting 3 mL of glacial acetic acid to two cotton balls in an 8 mL polypropylene vial (Thermo Fisher Scientific, Waltham, MA) with 3 mm bore size in the vial lid. Traps baited with different treatment blends of HIPVs in five replicates were assigned in four (Spain) or five (Hungary) rows, each containing treatments tested in a randomised block design. Traps were positioned 1.7 m above the ground in each trap tree and were spaced 20 m apart in each row. Trap position was **not** rotated in Spain (and in Hungary??)

Con formato: Superíndice

Con formato: Fuente: Negrita

Data Analysis

Comentado [CG2]: This analysis is not appropriate, as I indicated in the previous manuscript, but it is up to you

The variance of mean captures obtained with each compound or each treatment was stabilised using $\sqrt{(x + 1)}$ of counts for tests of significance of treatments using ANOVA. Significantly different treatment means were identified using Tukey's LSD at the 5% level (SAS Institute Inc. 1998).

Results

Trapping European grapevine moth in Spain with binary blends. Traps baited with phenylacetonitrile + acetic acid ~~significantly~~ captured significantly more male and female *L. botrana* than traps baited with acetic acid alone or blank traps (P <0.05). A total of one hundred and thirty-one males and females were captured in traps baited with phenylacetonitrile + acetic acid and the ratio of males to females was 1 : 0.7. There was no difference between male captures in traps baited with phenylacetonitrile + acetic acid and traps baited with indole + acetic acid. In all other treatments, there was no difference in the number of males and females captured than in traps baited with acetic acid alone or in blank traps.

Comentado [CG3]: I get 83 males and 48 females = 1:0.58 male:female ratio

140 *Trapping European grapevine moth in Hungary with binary blends.* Traps baited
141 with phenylacetonitrile + acetic acid significantly captured more male and female
142 *L. botrana* than traps baited with benzyl alcohol +acetic acid or acetic acid alone
143 (P <0.05). A total of forty-nine males and females were captured in traps baited
144 with phenylacetonitrile + acetic acid and the ratio of males to females was 1 : 0.5.
145 There was no difference between females captures in traps baited with
146 phenylacetonitrile + acetic acid and traps baited with 2-phenylethanol + acetic
147 acid. In all other treatments, there was no difference in males and females
148 captured than in traps baited with acetic acid alone.

149 **Discussion**

150 A binary blend of phenylacetonitrile + acetic acid significantly attracted more male
151 and female *L. botrana* than traps baited with acetic acid alone or blank traps.
152 Similar results were obtained with other ESBM and PLR (El-Sayed et al. 2016;
153 Suckling and El-Sayed 2017; El-Sayed et al. 2018). In contrast, the catch in traps
154 baited with other HIPVs compounds including, indole, 2-phenylethanol, benzyl
155 alcohol and (E)-nerolidol plus acetic acid was not significantly different from traps
156 baited with acetic acid alone. 2-Phenylethanol + acetic acid attracted large number
157 of males and females OBLR (El-Sayed et al., 2016). However the response of adult
158 EGVM to this binary blend was not different from acetic acid alone.
159 Phenylacetonitrile was not produced by grape berries infested with *L. botrana*
160 larvae in our preliminary analysis (El-Sayed et al. unpublished). Therefore, the
161 response to phenylacetonitrile and acetic acid suggests that male and female EGVM
162 can detect HIPVs by another herbivore. Possibly *L. botrana* would encounter these
163 compounds when grape leaves infested with leafrollers, for example, the

Sparganothis pilleriana (Denis & Schiffermüller) (Lepidoptera: Tortricidae: Tortricinae) which attacks grape leaves (Arn et al., 1988). El-Sayed et al. (2016) suggested that the production of HIPVs might indicate that host plants are more susceptible to attack than healthy plants. Accordingly, the positive response obtained in this study suggests that *L. botrana* can detect infestations by heterospecific caterpillars, possibly as a way to detect host plant susceptibility and oviposition sites.

Extension of the phenomenon from leaf feeding Tortricini to Olethreutinae was not obvious or expected, because *L. botrana* is mostly a direct pest of fruit, although it feeds on different vegetative parts: inflorescence, developing fruit and developed fruit (Ioriatti et al. 2011). In this study, adult *L. botrana* were attracted to a binary mixture of certain aromatic compounds together with acetic acid. Further improvements including ternary blends can now be investigated for this species. Results here suggest that new opportunities may emerge for pests undergoing geographic range expansion, and extend the phenomenon of attraction across species and subfamily boundaries. Fortunately, the compounds likely to be of interest here are widely available. A number of applications can be envisaged against *L. botrana*, particularly for use with mating disruption products. Pest managers will want answers to many questions, including trap efficiency, active space and other components, and we can predict incremental or better improvements with more complex mixtures of odourants. It seems likely that these new tools will be amenable to research and development in these areas for the first time with a number of pest Tortricidae. Comparisons and combinations with sex pheromones can also be investigated.

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Authorship Contributions

A.M.E. designed the experiments and analyzed the data; C.G. and J.J. conducted the field trapping experiments; A.S. help with lure preparation of the blend tested; A.M.E wrote the manuscript, while all authors contributed to discussion and the revision of the manuscript.

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Figure Legends

Fig. 1. Mean (±SE) of the total number of adult male and female ~~tortricids the EGVM (~~*L. botrana*~~)~~ caught in Spain in traps baited with binary blends containing 100 mg of each HIPV compound + 3 mL of acetic acid.

Comentado [CG4]: Don´t need to indicte mean+SEM since this is in the y-axis. Should be SEM not SE. Need to indicate meaning of letters. "Mean of the total number" is not a standard unit: The standard one Insects/trap/sampling date. See the attached doc about the problem with the data of Spain-compare with previous version of manuscript

Fig. 2. Mean (±SE) of the total number of adult male and female tortricids the EGVM (*L. botrana*) caught in Hungary in traps baited with binary blends containing 100 mg of each HIPV compound + 3 mL of acetic acid.

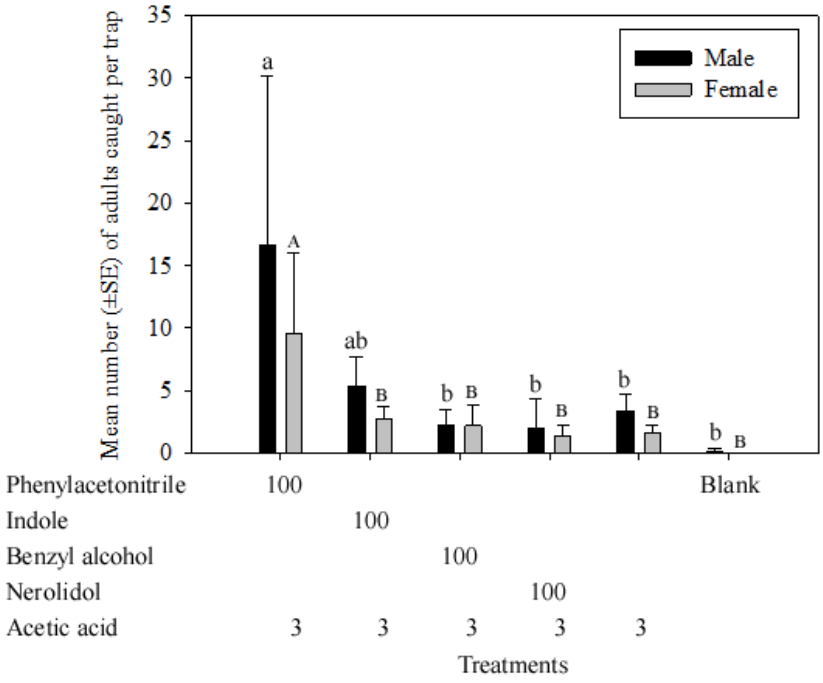
Mean number (±SE) of adults caught per trap
35
30
25
20
15
10
5
0
Male
Female
a
A
ab
B
b
B
b
B
b
B
b
B
Phenylacetonitrile 100 Blank
Indole 100
Benzyl alcohol 100
Nerolidol 100
Acetic acid 3 3 3 3 3
Treatments

Fig. 1

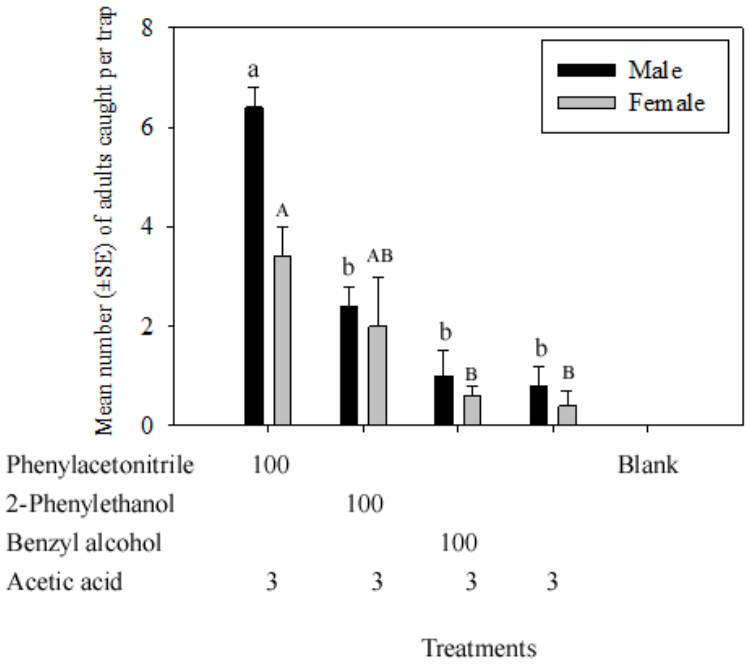
Mean number (±SE) of adults caught per trap
8
6
4
2
0
a
A
b
AB
b
B
b
B
Male
Female
Phenylacetonitrile 100 Blank
2-Phenylethanol 100
Benzyl alcohol 100
Acetic acid 3 3 3 3
Treatments

Fig. 2